## GENERAL INTRODUCTION

In 1998, the U.S. Environmental Protection Agency (EPA) requested that the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluate the validation status of the Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX) (**Appendix 9**). The EPA stated that this assay, developed to assess developmental toxicity, appeared to meet many of the ICCVAM validation criteria, and that it had been used in human health and water quality assessments. Possible regulatory applications for developmental toxicity identified by EPA included screening and prioritizing compounds for further testing, evaluating complex mixtures and environmental samples, and providing supplemental information in a weight-of-evidence evaluation of human developmental toxicity hazards. Stated advantages of FETAX included:

- a standardized test procedure;
- a published atlas of abnormalities;
- a database of over 100 compounds suggesting an overall accuracy for predicting mammalian teratogens of approximately 90%;
- the availability of mechanistic data indicating similarities between developmental toxicity in FETAX, laboratory mammals, and humans;
- the ability to test chemicals with and without a metabolic activation system (MAS);
- the ability to use the assay either in the laboratory or *in situ*; and
- an ability to evaluate single chemicals or complex mixtures.

In addition, based on multiple validation studies, test developers stated that FETAX appeared to be reproducible within and between laboratories. Stated possible limitations of the assay and areas requiring further discussion included:

- the appropriateness of the calculated TI for identifying negative and positive responses in the assay;

- the influence of the physico-chemical properties of environmental samples or exposures on the frequency of malformations in FETAX; and

- identification of appropriate applications for regulatory purposes and interpretation of data for human health purposes.

ICCVAM agreed to coordinate a review of the method. Subsequently, NICEATM was charged with preparing a BRD summarizing the available data and the extent to which each of the ICCVAM validation and acceptance criteria have been met (**Appendix 15**).

FETAX, which uses the embryos of the South African clawed frog (*Xenopus laevis*), was introduced in 1983 by Dr. James Dumont (Dumont et al., 1983). The assay was developed to evaluate the teratogenic and developmental toxicity potential of chemicals, metals, and complex mixtures (Dumont et al. 1983; Kamimura and Tanimura, 1986; ASTM, 1991; 1998; Sakamoto et al., 1992; Finch, 1994; Bantle, 1995). A number of inter-laboratory validation studies, largely directed by Drs. John Bantle and Douglas Fort, have been conducted to validate the utility of this assay for developmental hazard assessment. In this short-term *in vitro* assay, carefully selected, prepared (dejellied), and staged *X. laevis* embryos are exposed continuously to a test substance for the first 96 hours of embryonic development (ASTM, 1991; 1998). The primary endpoints assessed include mortality, malformations, and growth inhibition (ASTM, 1991; 1998).

The developers of this assay have proposed that data obtained using FETAX may be extrapolated to other species including mammals, may be used to prioritize chemicals and complex mixtures for further tests that use mammals, and may be used in ecotoxicological (e.g., water/soil/sediment)

hazard assessment (ASTM, 1991; 1998; Bantle, 1995; Fort et al., 1995; 1996b; Fort et al., 1997). Initial studies conducted using substances with known laboratory mammal and/or human developmental toxicity suggested that the predictive accuracy of FETAX, in the absence of an MAS, exceeded 85% (Sabourin and Faulk, 1987; ASTM, 1991). Furthermore, it has been proposed that inclusion of an MAS should increase the predictive accuracy of the assay for detecting substances with mammalian (including human) developmental toxicity to approximately 95% (ASTM, 1991; 1998).

This BRD presents an evaluation by NICEATM of the utility of FETAX for detecting potential human teratogens, and its use in water/soil/sediment developmental hazard assessment. The structure of the BRD follows the evaluation criteria guidelines found in the *Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to the Interagency Coordinating Committee on the Validation of Alternative Methods* (**Appendix 15**).